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Seminar

Transmissible spongiform encephalopathies

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Abstract

Nosologically, **transmissible spongiform encephalopathies** (TSE or prion diseases) should be grouped with other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, which are all caused by toxic gain of function of an aberrant form of a constitutively expressed protein. Failure to clear these proteins from the brain induces neuronal dysfunction. Transmissibility is the property that separates TSE from other neurodegenerative diseases, and this property seems to reside within the structure of the abnormal protein. The human phenotypic range of these encephalopathies includes Creutzfeldt-Jakob disease and its variant form, kuru, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia. Notwithstanding the generally low incidence of TSE and their limited infectiousness, major epidemics such as bovine spongiform encephalopathy and kuru arise in situations where intraspecies recycling of the abnormal protein is sustained. Moreover, evidence of chronic subclinical infection in animals offers insights into pathogenesis and prompts re-evaluation of the notion of species barriers and present infection control measures. Since case-to-case transmission is the only known mechanism underlying epidemics of TSE, potential reservoirs of infectivity in the tails of epidemics need continued vigilance.

Article Outline

Transmissible spongiform encephalopathies (TSE or prion diseases) form a biologically unique group of infectious fatal neurodegenerative disorders, which are caused by toxic gain of function in a normal host cell protein (the prion protein, PrP).[1] Human TSE include classic Creutzfeldt-Jakob disease (CJD) and the variant form, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia, and kuru. Fatal familial insomnia, Gerstmann-Sträussler-Scheinker syndrome, and familial CJD are genetically determined by mutations within the open reading frame of the PrP gene (*PRNP*).[1] Less distinct or overlapping neurological and neuropsychiatric syndromes, usually linked to specific mutations and polymorphisms within *PRNP*, contribute to an expanding spectrum of TSE.[2] These less common phenotypes are increasingly recognised because of greater use of specific confirmatory tests, including *PRNP* genotyping. Non-human TSE include scrapie of sheep and goats, bovine spongiform encephalopathy, and chronic wasting disease of mule deer and elk.[1] Recognition of variant CJD, zoonotically linked to bovine spongiform encephalopathy, has heightened awareness of this group of disorders. [3 and 4]

Molecular biology of PrP and pathogenesis of TSE

The pathogenesis of TSE is linked to simultaneous expression of normal PrP (PrP^{c} or PrP^{sen})

and accumulation of structurally aberrant, protease-resistant, conformers (PrP^{res}).[5] These protein conformers have identical primary structures (aminoacid sequences) but differ at a higher structural level such as folding. PrP^{res} is a generic term, denoting abnormal conformers associated with, for example, scrapie (PrP^{Sc}) and CJD (PrP^{CJD}). Nosologically, therefore, TSE should be grouped with other neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, which are also associated with aggregating conformers of constitutively expressed proteins, with soluble toxic species related to disease pathogenesis.[6] However, the infectiousness of TSE is a fundamental difference.

Normal PrP

PrP^{c} (figure 1) is encoded by *PRNP*, a small, single-copy, housekeeping gene on chromosome 20, which is expressed at highest levels in neurons.[7] The gene has only three exons and the entire open reading frame is in one exon. The human PrP^{c} protein is synthesised as a 253 aminoacid polypeptide chain from which the first 22 aminoacids (signal peptide) are cleaved shortly after translation commences. Post-translational processing adds a C-terminal glycosylphosphatidylinositol (GPI)-anchor at residue 230, which facilitates glycolipid linkage of PrP^{c} to the cell membrane. Two N-linked glycosylation sites are located at residues 181 and 197. A nonapeptide followed by four identical octapeptide repeats are normally located between residues 51 and 91. A normal polymorphism (valine or methionine) resides at residue 129 (figure 1).

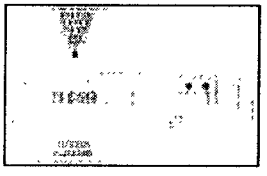

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Figure 1. *PRNP* mutations associated with familial forms of human TSENeuropathologically confirmed (red) and unconfirmed (blue) CJD mutations. Mutations associated with Gerstmann-Sträussler-Scheinker syndrome (green). *Associated with familial forms of CJD and fatal familial insomnia: the clinical phenotype of this mutation segregates with valine or methionine at residue 129, respectively.

Nuclear magnetic resonance analysis of recombinant PrP^{c} from various species suggests that all consist of three α-helical regions (two linked by a disulphide bridge), a short anti-parallel β-pleated segment, and a flexibly disordered N-terminus up to residue 120. Overall structure is that of a small globular protein.[8] Crystallographic studies lend support to this monomeric structure, but in the dimeric form, an unusual domain swapping of helix 3 is apparent, with creation of a novel short anti-parallel β-sheet segment at the molecular interface. [9] There is a high level of structural identity between bovine and human PrP. [10]

Although the biological function of PrP^{c} remains to be defined, it has been implicated in diverse activities, including neuronal copper metabolism[11] and synaptic transmission. [12] Polypeptides based on the PrP^{c} sequence selectively bind divalent copper ions,[11, 13 and 14] and this binding confers an ordered secondary structure. [13] Two binding regions have been delineated. [14, 15 and 16] Binding affinity has been described in the fmol/L [15] to μmol/L range, [17 and 18] with the latter seeming most probable and relevant to

physiological function. Binding of other divalent transition metals such as nickel, zinc, and manganese to PrP is less certain. How copper binding relates to normal PrP^c function and whether it has any role in disease pathogenesis remains to be clarified.[18] Nevertheless, copper binding aids restoration of resistance to proteinase K and disease transmissibility to PrP^{res}, which had lost these characteristics through partial denaturation.[19]

Generation of PrP^{res} from PrP^c

The primary aminoacid sequences of PrP^c and PrP^{res} are exactly the same. PrP^{res} is only detectable in the context of disease, developing through post-translational modifications that involve conformational rather than covalent change. Furthermore, the amounts of PrP mRNA transcripts in the brain do not rise as disease progresses.[7] Laboratory data, including repeated failure to detect a conventional infectious agent, have consolidated the protein-only (prion) hypothesis, that PrP^{res} constitutes predominantly, if not exclusively, the infectious unit in TSE.

PrP^c is directed to the endoplasmic reticulum by the signal peptide, where simple N-linked oligosaccharides and the GPI-anchor are added, and it arrives at the cell surface after transiting the Golgi apparatus where further oligosaccharide modifications take place (figure 2). Most PrP^c is transported to the cell surface where it is predominantly located in specialised detergent-resistant microdomains (DRM) known as rafts or caveolae.[20] Findings of transfected-cell studies indicate that wild-type PrP cycles between the cell surface and an early endocytic compartment, via an association with clathrin-coated pits, [21] but also can migrate to late endosomes or lysosomes via non-classic, caveolae-containing endocytic structures, apparently completely bypassing clathrin-related endocytic mechanisms. [20] Such variations in PrP^c endocytic trafficking could indicate the cell type in which exogenous PrP was expressed.[22] Disturbances in normal intracellular trafficking of PrP^c can culminate in its retrograde transport through the Golgi apparatus, with heightened accumulation of PrP^{res} in the endoplasmic reticulum.

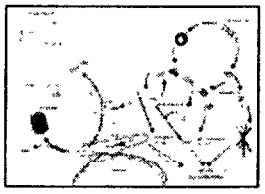
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Figure 2. Normal PrP^c synthesis and cell turnover with possible sites of conversion to PrP^{res}Mechanisms of toxicity and neurodegeneration remain unresolved.

The site of PrP^c to PrP^{res} conversion is uncertain. DRM[21] and the endosomal pathway [23] are possible sites for transformation. The endoplasmic reticulum may participate too, especially in familial TSE. [24] DRM could be important sites for initial PrP^{res} propagation during intercellular spread, because membrane-associated conversion seems to need insertion of PrP^{res} into the cell membrane, possibly by exchange of membrane particles or by GPI-anchor-dependent painting. Cell-free conversion models show the need for physical contiguity when different membrane components harbour PrP^c and PrP^{res}.[25]

Other aspects of normal PrP^c cell biology may be closely related to pathogenesis (figure 2). PrP^c has a half-life of only 5 h or so, and up to 10% of newly synthesised protein might be retrogradely transported from the endoplasmic reticulum to the cytosol, where it undergoes degradation,[26] although conflicting results have been reported. [27]

PrP^c synthesis followed by degradation and clearance of misfolded protein seem to be finely balanced functions, since incorrectly folded conformers are not detected under usual conditions. Manipulation of synthesis and degradation pathways has indicated possible mediators of PrP-related toxic effects and highlighted the complexity of the system. Perturbation of proteasome function results in wild-type PrP accumulation in the cytoplasm, which correlates with toxic effects in vulnerable cell lines and neurodegeneration in transgenic mice, without PrP^{res} formation.[28] However, findings of subsequent studies suggest that cytoplasmic accumulation of PrP^c may indicate an absence of translocation of the nascent PrP peptide to the endoplasmic reticulum under conditions of increased PrP expression rather than retrograde transport.[27] Nonetheless, PrP, harbouring mutations associated with familial TSE, accumulates in the endoplasmic reticulum [24] and cytoplasm in the absence of proteasomal inhibition. PrP accumulating in the cytosol forms aggregates, which acquire some properties of PrP^{res}, and, once present, persist despite only transient proteasome inhibition.[29] This occurrence suggests that PrP, by contrast with other proteasomaly degraded proteins, could have a unique innate ability to promote and sustain its own conformation change. Importantly, in-vitro toxic effects did not correlate with appearance of PrP^{res}.[29] Data of this type suggest a generic mechanism underlying age-related neurodegenerative diseases, wherein compromise of quality control of endoplasmic reticulum protein synthesis from whatever cause allows harmful soluble conformers to accumulate.

Once present, PrP^{res} seems to serve as a template for conversion of PrP^c to the abnormal disease-associated form, in a cyclic autocatalytic amplification, needing at least temporary dimerisation of the two isoforms. This template property of PrP^{res}, shown in a cell-free conversion assay,[30] has replicated in vitro many of the species and strain characteristics noted in TSE. The precise in-vivo mechanism by which PrP^c is converted to PrP^{res} remains to be clarified, but a stepwise transformation and acquisition of altered biophysical properties seems most likely, with folding intermediates, including molten globule forms.[21 and 31] An antibody to a highly conserved YYR motif of mammalian PrP has been identified, which selectively binds to the pathological isoform present in infected tissue. [32] Such approaches might help to dissect the structure of the pathological PrP isoform and the underlying structural changes. [33] Molecular chaperoning factors specific to the conversion process are also postulated. [34] In vitro, the two isoforms are reversible if buffer conditions are altered, but conversion rates can be slow [31] and limited under physiological conditions. [35] In solvent replacement studies, agents (formic acid, trifluoroacetic acid) most capable of converting β sheet structure to α helices were also the most effective at reducing infectivity.

PrP^c is soluble in mild detergents, protease sensitive, and shows a high α helical and low β sheet content.[36] PrP^{res} is only sparingly soluble in non-ionic detergents and shows relative protease resistance, a feature frequently exploited in diagnostic and experimental studies.[37] These properties directly relate to the enhanced β sheet content (>30%) noted in PrP^{res},[36] which also accounts for its innate amyloidogenicity with strong tendency to self-aggregation and fibril formation. [36] These properties have also prevented detailed structural analysis. The thermodynamic stability of PrP^{res} is noteworthy.

A key determinant of infectivity relates to the homology of PrP^c primary sequences (and consequent structures) in the donor and recipient species. Mouse and hamster PrP^c are 87% and 89% homologous with the human protein primary aminoacid sequence, with single aminoacid differences between mouse and hamster PrP^c effecting a species barrier.[38] Transmissibility could also be affected by secondary and tertiary structure identity between PrP species.

PrP^{res} fragments and glycotypes

Variations in tertiary structure of PrP^{res} probably correlate with differing surface exposures of the protein, and account for differences in cleavage sites with protease digestion. Proteinase K digestion of PrP^{res} removes a variable number of N-terminal aminoacids (up to around residue 90), and the resulting fragment shows a relative mobility of 27–30 kDa on western blots (PrP27–30).[5] Two nomenclatures have been used to describe protease-resistant PrP^{res} fragments in non-familial CJD. The first describes two principal PrP^{res} types after deglycosylation of PrP27–30, with electrophoretic mobilities of 21 kDa (type 1) and 19 kDa (type 2).[39] The second nomenclature describes four principal types that are distinguished by electrophoretic mobility, glycoform ratio, and effects of metal binding. [40 and 41] Detailed analysis of proteinase K digested PrP^{res} from a range of human TSE shows that the N-terminus can be cleaved anywhere from residue 74 to 102. In individual patients, there is most often a range of cleavage products, but predominance of digestion at glycine residue 82 arises in patients with type 1 PrP^{res}, whereas removal of aminoacids to the serine at position 97 is most abundant in type 2.

Is PrP^{res} solely responsible for neurodegeneration and infectivity?

20 years have elapsed since hamster-adapted scrapie infectivity was shown to copurify with a protein of 27–30 kDa (PrP27–30), and the term prion was coined to describe a proteinaceous infectious particle resistant to inactivation procedures that modify nucleic acids.[1 and 5] Considerable data now lend support to the primacy of PrP^{res} in disease pathogenesis and transmission. Nevertheless, some in-vitro and animal models of TSE prompt uncertainty.

Infectivity and neurodegeneration probably have separate mechanisms, and the role of PrP^{res} may differ. PrP^{res} is perhaps more directly related to transmission but, in the absence of PrP^c expression, PrP^{res} is insufficient to transmit disease or induce neuropathological changes.[42] Also, acquisition of one or more altered biophysical properties does not simply equate to transmissibility of disease. As yet, researchers have not been able to prove the protein-only hypothesis and induce a transmissible neurological disease with PrP^{res} generated by in-vitro conversion.[43] Doubt about the primacy of PrP^{res} stems from demonstration of minimal or no PrP^{res} in the brains of occasional patients and rodents manifesting—and even serially transmitting—disease.[44, 45, 46 and 47] For example, in bovine spongiform encephalopathy transmission studies to wild-type mice, [47] PrP^{res} was not detectable in brain homogenates of some mice despite successful transmission of a neurological disease. Generally, further passages did lead to emergence of PrP^{res}, and only then were spongiform change and overt gliosis present.

Insensitivity of biochemical assays might be relevant to absence of PrP^{res} despite disease transmission in rodents, but the situation seems more complex in some human genetic forms of TSE. Some patients dying from fatal familial insomnia,[45] and those with Gerstmann-Sträussler-Scheinker syndrome harbouring the A117V mutation, have no PrP^{res} detectable in their brains, and their disease is usually not transmissible. In patients with the A117V mutation there is instead upregulation of a transmembrane form of PrP (^{Ctm}PrP). Heightened expression of this abnormal form in transgenic mice correlates with spontaneous neurological disease, recapitulating many features of a TSE clinically and neuropathologically, but the disease is not transmissible.[44] ^{Ctm}PrP is also noted in non-genetic models of TSE. Negligible amounts of ^{Ctm}PrP are present in normal brain, despite facile synthesis of this and an N-transmembrane form in cell-free translation systems, in accordance with the hypothesis that altered topological forms and structural conformers of PrP are harmful and therefore usually degraded immediately after synthesis.[26]

Clinical spectrum of human TSE

Sporadic CJD

Sporadic CJD typically presents as a rapidly progressive dementia, often accompanied by cerebellar ataxia and myoclonus, with death in an akinetic-mute state after a median of 4–5 months. Around 90% of patients die within 12 months, although survival for more than 2 years is recognised.[48 and 49] Mean age at onset is about 60 years, with little difference in age-adjusted sex incidence. By striking contrast with the incidence of Alzheimer's and Parkinson's diseases, which rises sharply with age, that of sporadic CJD declines after age 70 years. [48 and 50]

Clinical features

Non-specific prodromal symptoms such as anxiety, sleep disturbance, and weight loss occur in up to 40% of patients in the days to weeks immediately before clearcut features of CNS disturbance. Very abrupt (even stroke-like) presentations over days are also recognised in up to 20% of patients. Cognitive decline and behavioural disturbance are invariable features, with myoclonus and cerebellar ataxia in 70–80% of cases.[49] Other extrapyramidal features, such as rigidity, develop in up to 50% of patients. Pyramidal dysfunction and brainstem disturbance with diplopia are not infrequent. Less common presentations include isolated disturbed vision (Heidenhain form), and non-myoclonic involuntary movements such as chorea, athetosis, and hemiballismus.

For sporadic CJD, the clinical profile and some key findings correlate with PrP^{res} glycoform type and *PRNP* codon 129 status.[51] Western blot PrP^{res} migration patterns (types 1 and 2), based on mobility of the unglycosylated band, coupled with presence of either valine or methionine at codon 129, allow delineation of six phenotypic subtypes. MM1 and MV1 correlate with around 70% of all cases of sporadic CJD. Electroencephalography usually shows typical widespread, synchronous, periodic, 1–2 Hz, sharp wave discharges and rapidly progressive dementia is the dominant clinical feature, with a mean illness duration of only 3·9 months. In patients with rarer subtypes, electroencephalography does not show diagnostically specific changes and survival is generally longer than 12 months. For example, MV2 patients present with ataxia, have kuru-type plaques, and the illness lasts an average of 17 months. MM2-thalamic patients show prominent thalamic and inferior olive degeneration, with little or no spongiform change, little PrP^{res} on western blot, and frequent insomnia—indeed, the clinical profile of this subtype has been designated sporadic fatal

insomnia.[52]

Clinical investigations

Electroencephalography (presence of periodic, 1–2 Hz, sharp wave discharges) previously was the only non-invasive investigation available to offer objective support for diagnosis, but its overall sensitivity is only 66–70%.[51, 53 and 54] Non-specific cerebrospinal fluid markers of neuronal injury are proving valuable, and the most useful ones are neuron-specific enolase and 14-3-3 proteins. [53, 55, 56, 57 and 58] In a large study, detection of 14-3-3 protein in cerebrospinal fluid proved superior to electroencephalography, with a sensitivity of 94% and a specificity of 84%, [53] which was similar to values in another study; [56] only rarely did patients manifest periodic, 1–2 Hz, sharp wave discharges but negative cerebrospinal fluid. [53] 14-3-3 protein in the cerebrospinal fluid has been incorporated into diagnostic criteria for sporadic CJD.

The limitation of 14-3-3 protein detection in sporadic CJD is non-specificity, and sensitivities and specificities of around 90% can only be achieved in highly selected groups, so this technique is best used for confirmation and not for screening. False-positive results are reported in a range of diseases causing substantial synchronous neuronal injury, including encephalitis (especially due to herpes simplex virus), recent cerebral infarction, and paraneoplastic neurological disorders.[53 and 56]

With MRI, typical findings in CJD and related disorders include high signal in the caudate nucleus and putamen.[59] Both fluid-attenuated inversion recovery and diffusion-weighted MRI sequences are preferred for patients with suspected CJD.

Genetically determined TSE

The three main genetically determined phenotypes are familial CJD, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia. All are inherited in an autosomal dominant pattern (figure 1). In these genetic forms, *PRNP* mutations predispose to production of PrP^{res} and high likelihood of disease during the individual's lifetime.

Familial CJD

Various *PRNP* mutations have been recorded in patients dying with CJD (figure 1), and these account for around 14% of the total. [60] In neuropathologically confirmed cases, several point mutations and octapeptide repeat insertions have been described. Concordance between mutation and phenotype is not absolute. Longer octapeptide repeat inserts are often associated with illnesses more in keeping with Gerstmann-Sträussler-Scheinker syndrome. [61] Additional mutations in cases not confirmed pathologically are missense changes, a single octapeptide repeat insertion, and a two-octapeptide repeat deletion. Phenotypic CJD diversity between and within affected families is recognised for specific mutations.

Absence of similar neurodegenerative disorders within a pedigree is commonly recognised for CJD-related mutations, raising the likelihood of high spontaneous *PRNP* mutation rates, [62] underappreciated family history, or incomplete penetrance, thus confirming the need for systematic genotyping if these disorders are to be properly classified. Penetrance of *PRNP* mutations is usually high, but existence of healthy octogenarian carriers of certain mutations suggests additional disease expression modifiers.[63] The E200K mutation has been identified as a founder effect for the high incidence of CJD recorded in certain geographic or ethnic subgroups. [63]

Gerstmann-Sträussler-Scheinker syndrome

The typical clinical features of this disorder are slowly progressive cerebellar ataxia, beginning in the fifth or sixth decade (but with onsets as early as age 25 years reported), accompanied by cognitive decline.[64] Patients with Gerstmann-Sträussler-Scheinker syndrome share the distinctive and defining neuropathological feature of widespread, multicentric amyloid plaques, which are immunoreactive for PrP. Experiments with non-human primates and rodents show Gerstmann-Sträussler-Scheinker syndrome to be transmissible, albeit with low overall take. Of *PRNP* mutations associated with the disorder, only the most common, P102L,[65] has been reproducibly associated with transmission, but even then in only 40% of such cases.

A spontaneous neurological disease with many features of human Gerstmann-Sträussler-Scheinker syndrome has been reported in a transgenic mouse model (P102L) of the disorder. [46 and 66] However, the low amounts of PrP^{res} and absence of multicentric amyloid plaques in this model prompt some reservations. Also, failure to develop spontaneous disease in a second murine model involving gene targeting to introduce a single copy of the mutant gene, [67] suggests that the neurological disease phenotype might need very high expression levels of the mutant gene in transgenic mice.

Genetics of Gerstmann-Sträussler-Scheinker syndrome and other less typical clinical forms

Since the initial description of the P102L mutation,[65] which remains the most common and confirmed in descendants of the original Austrian family, various *PRNP* mutations have been described for phenotypes of Gerstmann-Sträussler-Scheinker syndrome (table 1).[68] G131V and P102L mutations are generally associated with the typical clinicopathological profile of the disorder. Octapeptide repeats of varying size are noted in familial TSE; the shorter inserts are usually associated with CJD-like illnesses, whereas with typical Gerstmann-Sträussler-Scheinker syndrome, eight or nine octapeptide repeats are reported. The considerable clinicopathological diversity of this disorder is probably related, at least in part, to different *PRNP* mutations. Discordance within families points to additional genetic and environmental disease modifying factors, including codon 129 status.

Table 1. Less typical phenotypes reported in Gerstmann-Sträussler-Scheinker syndrome

 (18K)

Fatal familial insomnia

The term fatal familial insomnia was proposed in 1986 to describe an illness involving five members of a large Italian family.[69 and 70] In retrospect, the disorder probably should be classified as a type of thalamic dementia. [71] Sequencing of *PRNP* confirmed a mutation at codon 178, causing substitution of asparagine for aspartic acid (D178N). This mutation had been described in some familial CJD kindreds.[72] The apparently discordant phenotypes might be accounted for by the modifying effect of polymorphism at codon 129: fatal familial insomnia segregates with the D178N mutation when combined with methionine at codon 129, whereas familial CJD with D178N is linked at position 129 to valine. [73] Detailed studies of kindreds containing the D178N mutation have shown sufficient clinicopathological diversity and overlap to suggest that fatal familial insomnia and CJD represent distinct

profiles within a range of clinical patterns, and raise some doubts on the claim that the ultimate phenotype is governed entirely by the codon 129 allele. [62 and 74]

Clinical features

Onset is usually in the fifth decade, but ranges from age 20 to 63 years, with illness durations of 13–15 months (range 6–42).[45, 69 and 74] Non-specific symptoms, such as pronounced weight loss, can be an early feature as well as lethargy and tiredness. Incomplete penetrance may be common, but other studies have confirmed the occurrence of spontaneous germline mutations. [62]

Core clinical features are profound disruption of the normal sleep-wake cycle, with prominent insomnia, sympathetic overactivity, diverse endocrine abnormalities (particularly attenuation of circadian oscillations), and impaired attention. These features are thought to be related to severe selective loss of thalamic nuclei, with impairment of important integrative and relay functions between the cerebral cortex and brainstem within the limbic system and central autonomic network.

Clinical investigations

Neuroendocrine assessments typically disclose a range of hormonal irregularities. Serum cortisol concentrations are increased with or without preservation of the usual circadian pattern of secretion. Polysomnographic recordings confirm strikingly reduced total sleep time and gross disorganisation of sleep architecture on electroencephalography, including virtual absence of typical periods of rapid eye movement and deeper phases (non-rapid eye movement) characterised by K-complexes, spindles, and slow waves. Even drugs such as benzodiazepines and barbiturates may be unable to induce sleep-like electroencephalographic activity.

Detection of 14-3-3 proteins in the cerebrospinal fluid, diagnostically useful in sporadic CJD, [53] is usually absent in fatal familial insomnia. [75] Conventional neuroimaging with MRI or CT scanning is usually normal or shows non-specific cerebral or cerebellar atrophy, or both, whereas positron emission tomography with radiolabelled fluorodeoxyglucose often shows characteristic diminished metabolic activity in the thalami. [76]

Neuropathology

The brains of typical patients with fatal familial insomnia show characteristic restricted degeneration, largely confined to the thalami, especially the mediodorsal and anteroventral nuclei, and the inferior olivary nuclei.[69] As a result, in typical fatal familial insomnia, macroscopic examination of the brain generally does not show any gross abnormalities. [77] Irrespective of illness duration, low amounts of PrP^{res} are usually noted diffusely in the subcortical grey matter and the brainstem by immunoblot techniques, whereas it is infrequently detectable by immunohistochemistry, most often in a synaptic pattern or as small discrete deposits in the cerebellum and inferior olivary nuclei. In longer surviving patients, greater amounts of PrP^{res} are noted throughout the neocortex in association with more obvious and widespread spongiform change. Longer survival apparently allows more extensive and CJD-like topographical burden of disease. Of possible relevance to pathogenesis, the PrP^{res} glycoforms are both type 2 in sporadic fatal insomnia and fatal familial insomnia.[52]

Iatrogenic CJD

CJD can also arise from case-to-case horizontal transmission, invariably related to provision of health care. Iatrogenic CJD has arisen as a complication of neurosurgery, corneal grafts, implantation and therapeutic use of human dura mater, treatment with human cadaveric pituitary growth hormone and gonadotrophins, and stereotactic electroencephalography electrodes. Iatrogenic CJD remains rare, with 267 cases reported worldwide up to 2000.[78] Dura mater and pituitary growth hormone account for most cases. Generally, peripheral inoculation and dura mater implants are associated with ataxic presentations; with direct introduction of PrP^{res} into the cerebrum (including corneal grafts) the presentation is with dementia. Titre of inoculum and site of inoculation determine incubation period. Direct intracerebral contamination with PrP^{res} is associated with incubation times of only 16–28 months; with dura mater grafts, incubation can be 18 months to 18 years (median 6 years); the longest delays (5–30 years) are associated with subcutaneous injection of pituitary hormones.

Variant CJD

Variant CJD was first reported in 1996,[3] and subsequent biochemical, neuropathological, and transmission studies have substantiated initial concerns that the disease is zoonotically linked to bovine spongiform encephalopathy. [4]

Clinicopathologial features

The distinctive clinicopathological profile of variant CJD has allowed formulation of ante-mortem diagnostic criteria.[79] By striking contrast with classic CJD, patients with variant disease are much younger (median age at death 29 years), and about 60% present with psychiatric symptoms such as anxiety, insomnia, or withdrawal. [80] Neurological features are evident in about 35% of individuals at presentation, with unpleasant or painful sensory experiences the most common symptom. [80] By 2 months, nearly 60% of patients are reporting neurological symptoms, but it is generally more than 4 months before clearcut neurological signs such as gait disturbance, slurred speech, and tremor are evident, and longer than 6 months before involuntary movements (dystonia, chorea, or myoclonus), cognitive impairment, and ataxia are manifest. [80] Illness duration is usually longer than in classic CJD, with a median of 14 months. Death in an akinetic-mute state is a typical outcome. Neuropathologically, brains of patients with variant CJD harbour high burdens of widespread PrP plaques, some of which are encircled by vacuoles, prompting the designation florid plaques. All cases so far studied have shown MM homozygosity at the *PRNP* codon 129 locus,[79 and 80] a finding consistent with the pattern of susceptibility in kuru (see below).

Clinical investigations

The electroencephalogram does not show periodic, 1–2 Hz, sharp wave discharges in patients with variant CJD. Cerebrospinal fluid 14-3-3 protein is detected in only about half the cases, and tau protein in the cerebrospinal fluid may be more a more useful marker.[53 and 81] The so-called pulvinar sign (high T2 MRI signal in the posterior thalamus) is a useful distinguishing diagnostic feature, being present in about 75% of patients with variant CJD. [79]

Investigations with a very sensitive western blot technique have shown PrP^{res} to be present in the skeletal muscles and spleens of a few patients with sporadic CJD.[82] By contrast, the variant form is characterised by high amounts of PrP^{res} in, and transmissibility possible from, lymphoreticular tissues such as the tonsils and spleen, and, to a lesser degree, lymph nodes in

all patients.[83 and 84] This characteristic allows ante-mortem tonsil biopsy for diagnostic confirmation, but has created difficulties for public health because of the risk of human-to-human transmission through medical procedures, including use of blood and blood products. Data from a sheep bovine spongiform encephalopathy model confirmed transmission by intravenous blood transfusion, including from donor sheep midway through the incubation period. [85]

Other findings suggest greater secondary transmission risks for variant CJD than had been thought for the classic form. Retina and optic nerve were reported to have PrP^{res} by sensitive immunodetection, with amounts in optic nerve around 25% of those recorded in brain.[84] Low amounts of PrP^{res} were also detected in the rectum, thymus, and adrenal gland of one patient. Compounding concerns about the potential serial human passage of bovine spongiform encephalopathy are uncertainties about the existence of a large cohort of people infected with variant CJD but with long presymptomatic incubation periods, during which transmission is possible, as suggested by animal work.[86 and 87]

Kuru

Kuru came to the attention of western medicine in the mid-1950s.[88] The disorder was geographically circumscribed, endemic in the Fore linguistic group in the eastern highlands of Papua New Guinea, but was also seen in groups with whom the Fore often intermarried. [89] At the peak of the epidemic, an annual prevalence of up to 10% was seen in some Fore villages. [88 and 90] Kuru in the Fore language means to shiver. Along with cerebellar ataxia, these features form the principal clinical hallmarks of kuru. Classification as a TSE began when a veterinary pathologist noted epidemiological, clinical, and neuropathological similarities between kuru and scrapie, [91] followed by successful transmission of kuru to chimpanzees. [92]

Laboratory and epidemiological data pointed to transmission of kuru via cannibalistic rituals as part of the mourning for deceased relatives.[89] Women and children ate the internal viscera (including the CNS); men consumed less infectious tissues, such as skeletal muscle, and were at lower risk of kuru. Aside from ingestion of infectious tissues, conjunctival, nasal, and skin contamination were other possible modes of transmission. [93]

Since cessation of cannibalism in the mid-to-late 1950s, prevalence of kuru has steadily declined, although the epidemic tail is lasting longer than expected. The gradual tapering of the epidemic has been associated with a progressive increase in age at onset, with kuru victims in recent times aged older than 40 years. Contributing to this factor are susceptibility factors, age of exposure, and titre of ingested tissues. Methionine homozygosity at codon 129 of *PRNP* is one important susceptibility determinant, increasing the risk of kuru, with resultant younger age at onset, shorter incubation periods, and shorter illness duration.[94 and 95] Typically, MM children consuming highly infectious brains died from kuru before adulthood. Only later in the epidemic did valine homozygotes and methionine/valine heterozygotes, and those exposed to less infectious tissues, develop disease, usually in adulthood. Incubation periods of more than four decades are now recognised for the tail of this epidemic, which has had a major effect on the population genotypes of surviving Fore (table 2), and indeed suggests that other major epidemics of a TSE-like illness may have happened in prehistoric times to shape the worldwide distribution of this *PRNP* polymorphism.[96]

Table 2. Distribution of *PRNP* codon 129 polymorphisms in people with kuru, sporadic CJD,

and variant CJD compared with normal population frequencies

 (10K)

Chronic subclinical infection

The notion of latent or subclinical infection in TSE has been revived[98] by studies with mice. [86, 87, 99 and 100] A striking feature is the extended survival and apparent good health (for up to hundreds of days) despite chronic infection. Brains of these asymptomatic animals carry high titres of infectivity similar to those of mice dying from terminal disease, with typical spongiform changes sometimes seen histologically and PrP^{res} recorded on western blots.[86, 87, 99, 101 and 102] Such findings challenge previous ideas of the species barrier and the notion that disease expression is a prompt and inevitable result of PrP^{res} accumulation in susceptible hosts, and might also relate to observations of incomplete penetrance in older people carrying *PRNP* mutations.[69] Further, these data accord with hitherto unrecognised or underappreciated adaptive or compensatory mechanisms (such as enhanced clearance of misfolded or aggregated proteins) existing within mammalian cells, which could offer novel therapeutic strategies. If confirmed, these results would mean that highly sensitive biochemical assessments and masked transmission studies from apparently unaffected animals would have to be done before any particular test sample could be said to be free of infectivity.

The possibility of natural chronic asymptomatic infection engenders concerns about potential subclinical reservoirs that, at least theoretically, could relate to maintenance of low-level endemicity and the protracted tails seen after epidemics of TSE, and be relevant to the chance of secondary transmissions arising in health-care settings from people carrying subclinical disease. Unfortunately, no reliable, minimally invasive, specific marker for preclinical diagnosis is available.

Sporadic CJD and epidemics of TSE

CJD can arise de novo as a result of mutations in *PRNP*, be secondary to horizontal (case-to-case) transmission, or most usually (around 85% of total) be without apparent cause (sporadic). Sporadic disease has an annual incidence of only 1·0–1·5 per million,[48, 103 and 104] although unexplained rates up to 3·9 per million have been reported for Switzerland. [105] Such an unusual incidence may not simply be a result of enhanced ascertainment but could relate to exposure to bovine spongiform encephalopathy resulting in a sporadic CJD-like phenotype in *PRNP* codon 129 methionine homozygotes.[100] For sporadic disease, however, the usual explanation is spontaneous somatic mutation in the neuronal *PRNP* pool or rare stochastic conformational changes in expressed PrP^{c}.[1] Covert low-level contamination (especially in a health-care setting) could offer an alternative explanation for some cases. [106 and 107] Spatiotemporal clusters of CJD have been reported but point sources or case-to-case transmission links have not been proved. [108] By contrast with variant disease, little experimental evidence exists for blood as a vector for sporadic CJD, and findings of large case-control studies militate against this concern. [106]

Notwithstanding the low incidence of TSE and their limited infectiousness by comparison with many viral and bacterial diseases, epidemics such as bovine spongiform encephalopathy, variant CJD, and kuru (Box panel) can develop in unique situations. The precise origin of the bovine spongiform encephalopathy epidemic remains contentious.

Nevertheless, there seems little doubt that intercurrent with changes in rendering and livestock feeding practices (including supply of ruminant-derived protein supplements to calves), animals harbouring a TSE were recycled to healthy cattle with resultant rapid amplification to epidemic proportions. To date, nearly 200000 cattle have been identified with bovine spongiform encephalopathy in the UK, with spread to several other European countries, the Middle East, and Asia.[109 and 110] Back-calculation modelling suggests almost 2 million cattle have been infected with the disease to date, with 1·6 million likely to have entered the human food chain. [110]

Box panel. Epidemics of TSE on a low-level endemic background of scrapie and sporadic CJD

Countermeasures have curtailed the bovine epidemic and limited contamination of the human food chain. From December, 2000, European Union countries have been required to screen brains of all cattle older than 30 months for presence of PrP^{res} before human consumption of the animal is permitted. New in-vitro techniques are as sensitive as bioassays using transgenic bovinised mice (which themselves are 10000 times more sensitive than bioassays with some wild-type mice),[111] and these tests could prompt re-evaluation of earlier assessments of the transmissibility of these spongiform encephalopathies from non-CNS bovine tissue, especially with reports suggesting that certain murine skeletal muscles can propagate infectivity. [112]

As of Dec 1, 2003, 143 definite or probable cases of variant CJD (with 137 deaths) had been confirmed in the UK, with six in France, and one each in Italy, Canada, Ireland, and the USA.[113] All those tested are codon 129 methionine homozygous (table 2). The French and Italian patients possibly contracted the disease by eating exported UK beef products; the other three patients from outside the UK had been in the country during the probable peak exposure years of 1988–90. Despite continuing uncertainty, some reassurance is being taken from UK surveillance data [114] (figure 3) and results of modelling studies.[115 and 116] If assumptions are made, including an exponential fall in risk after age 15 years, the variant CJD epidemic may already have peaked, at least among codon 129 methionine homozygotes. These surveillance data, looked at with reference to the years that bovine spongiform encephalopathy entered the human food chain, suggest an average incubation period of around 12 years, and raise concerns about the possibility of a second wave of variant CJD as a result of human-to-human haematogenous transmission (figure 3).

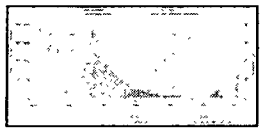 (26K)

Figure 3. Temporal profile of entry of bovine spongiform encephalopathy-infected cattle into human food chain and subsequent profile of reported variant CJDvCJD=variant CJD. cCJD=classic CJD. Incidence refers to absolute number of cases in every year. Temporal profile for bovine spongiform encephalopathy based on a back calculation differential mortality model

[110] (left axis). Profile of reported variant CJD (right axis) might have reached an initial peak in the cohort infected by the oral route. [114]

Decontamination issues

The infectious agents of TSE resist conventional sterilisation and decontamination methods, [117] especially on stainless-steel surfaces. [118] Mild (especially non-ionic) detergents, chlorine dioxide, alcohols, potassium permanganate, hydrogen peroxide, aldehydes, ultraviolet irradiation, and ethylene oxide are ineffective. Autoclaving at 134°C for at least 18 min in a porous load device or for about 1 h at standard autoclave temperatures in a gravity displacement steriliser, or soaking instruments in 1 mol/L sodium hydroxide or concentrated sodium hypochlorite (more than 5000 parts per million available chlorine) for 1 h, are the recommended procedures for reducing infectivity. [119] Enzymatic proteolytic inactivation methods (alone or in combination with detergents) are under development.

Therapeutic approaches

No proven treatment for human or non-human TSE exists. Research tends to focus on compounds postulated to prevent—directly or indirectly—misfolding of PrP^{c} to PrP^{res}, diminish neurotoxicity, or promote clearance of pre-existing PrP^{res}. Compounds studied include polyanions, sulfonated dyes, tetrapyrroles, polyene antibiotics, branched polyamines, cysteine protease inhibitors, acridine derivatives, phenothiazines, suramine, synthetic peptides,[120] and small interfering RNA duplexes, which have been shown to silence prion protein expression and transiently abrogate PrP^{res} accumulation in scrapie-infected neuroblastoma cells.[121]

Two common screening techniques for identification of potential therapeutic agents are chronically infected mouse neuroblastoma cells and in-vitro cell-free conversion assays. Reduction or elimination of detectable PrP^{res} from scrapie-infected neuroblastoma cells or scrapie-infected brain homogenates has sometimes correlated with prolongation of incubation times in disease models. However, this association does not always hold—for example, quinacrine cleared PrP^{res} from infected neuroblastoma cells but did not inhibit conversion in the cell-free conversion assay, suggesting that this drug does not act by directly preventing PrP^{res} formation.[122] Confirmation of this tissue culture result led to support for use of quinacrine in clinical trials. [123] However, failure of the drug to prolong survival in an in-vivo model of mouse-adapted TSE [124] or to reduce PrP^{res} load in spleens of intraperitoneally inoculated mice[125] showed that conditions in vivo are probably much more complex than those in tissue culture. Efficacy is generally inversely proportional to time since inoculation; survival is rarely prolonged substantially if the gap between inoculation and treatment is more than a week.

The ability of antibodies against PrP to clear PrP^{res} from infected cell cultures[126] and indefinitely prolong survival in peripherally inoculated mice, [127] coupled to success of this approach in clearing plaques in animal models of Alzheimer's disease, raises the possibility of a conventional vaccine approach. The generally poor immunogenicity of PrP^{res} (a native cellular protein with altered conformation) is discouraging. Nonetheless, a transgenic mouse model engineered to produce antibodies to PrP, which resists challenge to peripheral infection with scrapie,[128] lends support to future efforts in this direction. Finally, since PrP knockout animals seem healthy and are totally resistant to disease, therapeutic strategies

aimed at gene targeting *PRNP* for downregulation are being pursued.

Conflict of interest statement

None declared.

Search strategy and selection criteria

References cited were from the Creutzfeldt-Jakob disease bibliography, Department of Pathology, University of Melbourne, maintained by the Australian National Creutzfeldt-Jakob Disease Registry (http://data.path.unimelb.edu.au/RIS/RISWEB.ISA [accessed Sept 22, 2003]). Generally, references chosen fulfilled one or more of the following criteria: major breakthroughs in transmissible spongiform encephalopathy research; first reports of key observations, if replicated; largest studies reported to date, if more than one on a similar theme; offered comprehensive recent review (to save citing many original studies); or the most thorough or up-to-date study of a particular topic.

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